

REMARKS

With entry of the present amendment, claims 19 – 21 and 26 – 51 are pending. Claims 1 – 18 were previously canceled. Claims 32 – 40, directed to the non-elected invention are presently canceled without prejudice, along with claims 22 - 25. Claims 19, 20, 21 and 28 have been amended, and claims 41 – 51 are new. Pending claims 19, 47 and 49 are independent claims. New matter has not been introduced by the new or amended claims.

Claim 19 has been amended to recite that the specified genes are introduced genes, the dehydratase activity is a glycerol or diol dehydratase activity and that the gene encoding protein X is isolated from a glycerol dehydratase gene cluster or from a diol dehydratase gene cluster wherein protein X has no enzymatic activity. Support is found in original claims 22 and 23. Claim 20 has been amended to recite that protein 1 has an amino acid sequence of at least 95% similarity to SEQ ID NO:60 or SEQ ID NO: 61, protein 2 has an amino acid sequence of at least 95% similarity to SEQ ID NO:62 or SEQ ID NO:63; and that protein 3 has an amino acid sequence of at least 95% similarity to SEQ ID NO:64 or SEQ ID NO:65. Support is found at page 12 of the disclosure. Claim 21 has been amended to clarify that the host cells used for introduction of a gene were from the recited genera. Dependency has been changed in claims 24 and 25. Claim 28 has been amended in accordance with the Examiner's suggestion. The claim recites, the gene encoding protein X "consists of nucleotides" 0749 - 11572 of SEQ ID NO:19.

New claim 41, which depends from claim 19 further defines the carbon substrate and support is found at page 14, lines 10 - 15. New claims 42 and 51, which depend from claim 21 and claim 49 respectively, further define the recombinant microorganisms as an *E. coli*, a *Klebsiella spp.* or a *Saccharomyces spp.* Support is found at page 19, lines 26 - 27 of the specification. New claim 43, which depends from claim 19, further defines the gene encoding the glycerol-3-phosphatase as a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO:17, SEQ ID NO:33; and/or enzymatically active fragments thereof. Support is found at page 18, line 33 of the disclosure. New claim 44, which is depends from claim 19 further defines the gene encoding the dehydratase activity as a glycerol dehydratase of *Klebsiella pneumoniae*. Support is found at page 10, lines 25 - 33. New claim 45, which depends from claim 19,

further defines the gene encoding protein X as a nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 66 or SEQ ID NO: 59. Support is found in the description of the drawings and specifically Figure 2 and Figure 8. New claim 46 directed to protein X encoded by the ORF Z of the dha regulon of the genus *Citrobacter* finds support at page 11, lines 9 - 11.

New independent claim 47 is directed to an *E. coli* capable of producing 1,3 -propanediol from a carbon source comprising an introduced gene encoding a glycerol or diol dehydratase enzyme; and an introduced gene encoding protein X of a glycerol or diol dehydratase gene cluster wherein the carbon source is a monosacchride, an oligosaccharide, a polysaccharide, a one-carbon substrate and/or mixtures thereof. Support is found *inter alia* at pages 9, 11 and 14.

New independent claim 48 is directed to a microorganism capable of producing 1,3-propanediol from a carbon source comprising a) at least one introduced gene encoding a glycerol or diol dehydratase activity; and b) at least one introduced gene encoding protein X, wherein the gene encoding protein X (1) consists of nucleotides 0749 - 11572 of SEQ ID NO:19; (2) is an isolated nucleic acid molecule that hybridizes with (1) under the following hybridization conditions 0.1 x SSC, 0.1% SDS at 65°C; and (3) an isolated nucleic acid molecule that is completely complementary to (1) or (2), wherein production of 1,3-propanediol is greater in the recombinant microorganism than in the absence of said nucleic acid encoding protein X. Support is found *inter alia* at page 6, lines 5 - 15; page 11, lines 9 - 14 and page 15, lines 16 - 19.

New claims 48 and 50, which depend from claim 47 and 49 respectively, define the host microorganism as further comprising at least one foreign gene encoding a protein selected from the group consisting of protein 1, protein 2 and protein 3, wherein said protein 1 has an amino acid sequence of at least 95% similarity to SEQ ID NO:60 or SEQ ID NO:61, said protein 2 has an amino acid sequence of at least 95% similarity to SEQ ID NO:62 or SEQ ID NO:63; and said protein 3 has an amino acid sequence of at least 95% similarity to SEQ ID NO:64 or SEQ ID NO:65. Support is found at page 12, lines 12 - 25 of the disclosure.

Previous Objections and Rejections -

Applicants acknowledge the withdrawal of objections to the specification concerning (a) Figures 9 and 10; (b) the abstract; (c) use of the symbol ®; (d) the

description of protein X; (e) the address of the American Type Culture Collection; and (f) the spelling error in claim 24 (See points 1.1, 1.2, 1.3, and 1.4 of the Office Action dated October 6, 2003).

Additionally Applicants acknowledge the withdrawal of rejections previously made under 35 U.S.C. §112, second paragraph of claims 19 and 20 and claim 28 (See point 2.1 of the Office Action dated October 6, 2003).

Rejection under 35 U.S.C. §112, second paragraph.

The Examiner has rejected claims 19 - 31 under 35 U.S.C. §112, second paragraph. Specifically, the phrase "The recombinant microorganism of Claim 19 selected from the group consisting of *Citrobacter*, *Enterobacter*, etc" in claim 21 and the phrase "as shown between positions 0749 - 1157 of SEQ ID NO:19" in claim 28, are alleged to be confusing. Further, claims 29 - 31 have been rejected because the limitations "protein 1", "protein 2", and "protein 3" lacked antecedent basis. Additionally, the Examiner contends the term "comprising" in the preamble of claim 19, leaves open to interpretation of whether or not "a) at least one gene encoding a dehydratase activity, b) at least one gene encoding a glycerol-3-phosphatase and c) at least one gene encoding protein X" are native genes or if they were introduced. As amended claim 19 clearly recites that the genes are "introduced" with respect to the recombinant microorganism.

Applicants contend the amendment to the claims renders moot each of the stated rejections and withdrawal of the rejections is requested.

Rejection under 35 U.S.C. §112, first paragraph.

The Examiner has alleged claims 19 - 31 fail to comply with both the written description requirement and the enablement requirement of section 112. Applicants contend the pending claims and new claims comply with both requirements.

Subgenus (a) of claim 19 is now directed to genes encoding glycerol or diol dehydratases. Specific glycerol dehydratases and diol dehydratases are taught in the specification, for example GenBank U09771 and U30903 and diol dehydratase (Gen Bank D45071). Also these enzymes are disclosed as being classified in E.C. 4.2.1.30 and E.C. 4.2.1.28 (See for example pages 2 and 10 of the disclosure). Subgenus (b) is directed to the enzyme G3P and this enzyme is taught as being encoded by the GPP1

and GPP2 genes (GenBank Z47047 x125 and GenBank U18813x11) (See for example page 3, 13 and 14 and 18 of the disclosure). Not only do Applicants provide a function and a structure for the recited enzyme activities but also the specification discloses that one skilled in the art recognizes that sequences encompassed by the invention are also defined by their ability to hybridize under stringent conditions with the exemplified sequences.

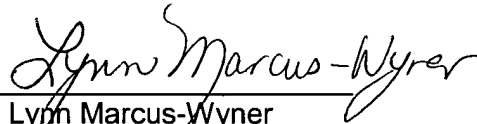
With respect to subgenus (c), amended claim 19 is directed to protein X isolated from a glycerol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella* and *Citrobacter* or ii) isolated from a diol dehydratase gene cluster from an organism selected from the genera consisting of *Klebsiella*, *Clostridium* and *Salmonella*.

The alignment of the amino acid sequences of protein 1, protein 2 and protein 3 in figures 3, 4, and 5 of *Klebsiella pneumonia* and *Citrobacter freundii*, and the language of similarity at page 12 of the disclosure supports the "at least 95% similarity" recited in the claims. The variant of protein 1, protein 2 or protein 3 having the disclosed sequence must in combination with protein x retain the characteristics of increasing the production of 1,3 propanediol in a host over the production of 1,3 propanediol in the host in their absence (See page 12 of the disclosure).

It is believed that the claims, as currently amended, are in condition for allowance and reconsideration is respectfully requested. If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 846-7620.

Respectfully submitted,

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